Ralstonia solanacearum in Geranium (*Pelargonium* sp.): Testing and Sampling Plan for Off-shore Facilities Shipping to the US

Background:

In February 2003, the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) confirmed the presence of *Ralstonia solanacearum* race 3 biovar 2 in geraniums in four greenhouses in Illinois, Indiana, Michigan, and Wisconsin. This is the first time this pathogen has been detected in the United States since 1999. *R. solanacearum* race 3 biovar 2 is a bacterial pathogen that causes common wilt in geraniums and infects numerous solanaceous plants (e.g. tomatoes, eggplant, tobacco, and peppers). It is also a major concern to the potato industry because the disease survives well in cold temperatures and renders potatoes unmarketable.

In 1999, *R. solanacearum* race 3 biovar 2 was confirmed in commercially grown geraniums in green houses, confined to the states of WI, NY, NJ, PA, and SD. The pathogen was traced back to infected geraniums that were shipped to US from greenhouses in Guatemala. Pathway analysis revealed the mode of entry of *R. solanacearum* race 3 biovar 2 into the US is through four states (FL, CA, NY, and TX), with the major ports of entry located at Miami, San Diego and LA, JFK, and El Paso and Houston.

In February 2003, *R. solanacearum* race 3 biovar 2 was identified in greenhouses in the US. Trace back investigations conducted by PPQ have determined that the origin of *R. solanacearum* race 3 biovar 2 was imported geranium from Kenya. In the United States. *R. solanacearum* race 3 biovar 2 is a serious concern for the potato industry because of the cold tolerant nature of this plant pathogen, and the potential for its establishment and spread into major potato producing areas.

In an attempt to prevent the entry of this plant pathogen into US, APHIS PPQ will institute the following procedures for sampling and testing of host material in the country of origin before it can be eligible for entry into USA.

Sampling of geranium host material

Phytosanitary certification and eligibility for the additional declaration requires samples of geranium mother plants be taken under authority of the Ministry of agriculture. Samples should consist of **all suspect wilted plants** and should be taken throughout each greenhouse in the production facility. Samples should be taken weekly and officials must

sample the whole plant including roots without the soil, if possible. Bare root plants are ideal. **Since the pathogen is concentrated in the lowe r stem, the disease may not be detected from samples if only leaf or partial stem samples are taken.**

Serological Testing

Testing of samples may be done by a variety of approved lateral flow and strip serological test kits. Instructions for the operation of the tests should be followed as specified in the manufacturers instructions provided with each kit. Please read all materials associated with the kits and the kit components to verify proper storage of the reagents and flow devices. Check the manufacturers instructions for each new shipment since instructions may be modified to improve the performance at any given time.

i) ***Rs* ImmunoStrip Test**
Agdia, Inc
30380 County Road 6
Elkhart, IN 46514
www.agdia.com
Ph. 800-622-4342
FX 219-264-2153

ii) **Potato Brown Rot Pocket™ Diagnostic**
Central Science Laboratory (CSL)
Sand Hutton, York, YO41 1LZ
www.csl.gov.uk
Ph 44 1904 462600
FX 44 1904 46211

iii) ***Ralstonia solanacearum* SPOT vCHECK LF™**
ADGEN, LTD.
Nellie's Gate, AYR
Scotland, KA6 5AW
www.adgen.co.uk
Ph 44 1292 525275
Fx 44 1292 5255477

Method of kit selection and observations

APHIS has tested the kits listed above as follows:
The tests were run as directed by the manufacturer's instructions provided in the kits. We recommend that these kit instructions are followed while performing the tests. All kits require use on symptomatic plants, and for use on stem sections and segments, we followed those instructions. To conduct these tests plant material was selected, chopped and mixed to distribute potentially infected material within the sample, and then the appropriate amounts of plant materials were distributed to the test vials to conduct the test. The control plant material was healthy and *Ralstonia solanacearum* – infected geranium.

All three tests detected the bacterium in infected plants and did not react to healthy plants. There was a slight difference in sensitivity of the tests based on intensity of the band on the flow device.

We found the consistently darkest and easiest test bands to read was the CSL Potato Brown Rot Pocket Diagnostic test kit. The CSL test was consistently the fastest reacting (instant reaction to 3 minutes) and produced the darkest test bands that were the easiest to score

The Agdia *Rs* ImmunoStrip test kit, in early testing, produced a lighter test band, however modifications to the ACL strip has resulted in the production of darker bands on the flow device that make them easier to read. The Agdia test produced consistently medium dark to dark test bands read in about 10-30 minutes and were generally easy to score.

The ADGEN *Ralstonia solanacearum* SPOT vCHECK LF™ also detected infected positives, but in one test the band was so faint that it may have been missed. The test bands and control bands in the SPOTCHECK system were generally lighter than the other two tests, yet they were generally consistent with the CSL and Agdia systems in detecting a positive. The ADGEN test produced medium dark to faint test bands instantly to 3 minutes, and in a few cases were hard to score.

In summary, all three tests will detect the pathogen, however, in our hands using samples with a varying range of quality, we recommend the CSL and Agdia tests for a wide range of samples. The ADGEN test is useful for samples that are of good to fair quality and not of fair to poor quality.

Race 3 biovar 2 determination.

If a sample tests positive for *R. solanacearum*, the producer may choose to not export from that facility and no further testing is necessary. If, however, the exporter wants to identify the pathogen to race and biovar, they must use a PCR procedure. Please contact APHIS-PPQ for recommendations on the PCR procedure to follow.

If *R. solanacearum* race 3 biovar 2 is confirmed the greenhouse will be disqualified from export until mitigations adequate to eradicate the organism. Eradication method must be approved by APHIS-PPQ. Please contact APHIS-PPQ to discuss eradication efforts.

If PCR is not conducted, and an approved serological test indicates the presence of *R. solanacearum*, then every effort must be made to eradicate the pathogen by an APHIS-approved method. Please contact APHIS-PPQ to discuss eradication efforts.

Weekly testing by the plant protection organization of the country of origin or their designee for *R. solanacearum* will be required before facilities can be certified to ship host material to the United States. Weekly testing will continue throughout the shipping season. APHIS may also require approved certification of cultural practices and periodic inspection of facilities during periods of production and testing.

APHIS reserves the right to examine facilities that have tested positive for *Ralstonia solanacearum* prior to reestablishment of exports of host material to USA.

MINIMUM SANITATION PROTOCOLS FOR OFFSHORE GERANIUM CUTTING PRODUCTION

APHIS has developed protocols for safe sanitation practices that will be required for future shipments of geraniums exported to the United States. These protocols were developed and discussed with exporting countries and the companies affected.